

pMC9 Revisited:

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Progress: To repeat the exercise again with pMC9 for fidelity

Just :: to titrate the amount of template I used 100 - 200 pg / 35 cycles / 3 step 3' extension - but of mispriming was obvious. Today has used earlier less template / 30 cycles / 2 step less of other products 5' extension

so tried 0 10 25 50 100 & 200 pg of starting template / 5' extension / 2 step

with 1 U of enzyme product yield is very little - but with 2 U of Tag quite a good product yield was obtained.

so tried with both 1U & 2U of Tag alone.

To start with, in this expt Tag, DV wasn't included

Conditions:

10x buffer K-T.

94°, 30"

200 µM dNTP

.4 µM primers (non-dV)

30 (94°, 30", 68°, 5')

2 mM Mg

Template 0 - 200 pg

enzyme 1 & 2 U.

- prepared a premix 12 x 45 µl

7 tubes: 1 - 12 1U
13 - 24 2U

- added 5 µl of different amounts of template

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Used & Understood by me,

Date

1/9/95

Invented by

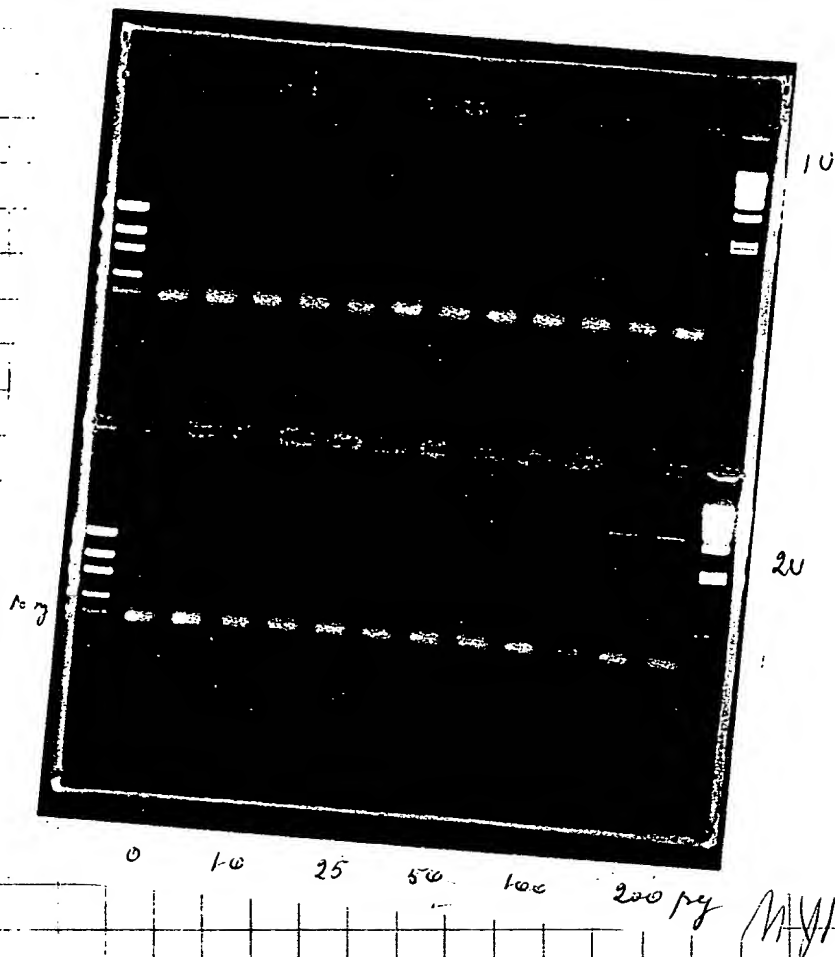
Date

Record d by

K. Subraman

12/19/94

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TagResult:

200 pg / 10 enzyme ✓

25 pg up / 12x product
 obtained but
 rather very
 yield even with
 200 pg of starting
 template.

~ 5 - 10 ng / 8 pl

= 31.25 - 62.5 ng / 8
 from 200 pg temp

~ 300 fold ampl

~ 8 doublings!

* Try 50 pg } 20 200 pg - 10
 200

- cycles increase to 35 atleast

- go up to 50, 60 -

- cycling regime looks ok - gives cleaner product

no mispriming seen

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Witnessed & Understood by me,

Date

1/9/95

Invented by

Recorded by

K. Sitarman

Date

12/20/94